Table 2 Examples of discontinuous buffer systems with constant trailing ion concentration[a]

Trailing ion species	*Concentration of trailing ion species* (mmol L^{-1})	*Ion concentration* (mmol L^{-1})	*Net mobility* ($\times 10^4$ cm^2 V^{-1} s^{-1})	*Trailing zone conductance* (cm^2 Ω^{-1} mol^{-1})	*Leading ion concentration* (mmol L^{-1})
Hepes	31.6	30	1.38	1.17	60
Tricine	37.2	30	1.76	1.38	55
Asparagine	49.4	30	1.70	1.56	65
Glycylglycine	41.5	30	2.06	1.58	65
Taurine	45.2	30	2.17	1.70	55
Glycine	138.5	30	0.81	1.83	160

[a] The leading ion in all cases is formate (ion mobility of 5.50×10^{-4} cm^2 V^{-1} s^{-1}) at the indicated ion concentration. The counterion in all cases is Tris (ion mobility of 2.60×10^{-4} cm^2 V^{-1} s^{-1}).

complexity of the technique or ignorance of the technique's advantages. However, the benefits of sample stacking, mobility tailoring and an ionic reference front are unchanged and unique when compared to zonal buffer systems. With new applications and challenges for electrophoretic separations, renewed attention to the technique is certain.

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Electrochromatography

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Introduction

The application of electric fields to planar chromatographic media in order to drive and/or enhance separations is as old as planar chromatography itself. There is evidence to suggest that paper electrophoresis was first performed several years prior to the first reports of paper chromatography. Research in the field has been intermittent, with periods of considerable activity separated by long periods of inactivity. This is at least partly due to attention being diverted away from planar methods to modern high-efficiency column techniques. It is quite conceivable that modern chromatography would be very different, with a much stronger focus on planar techniques, had more development work been carried out on thin layer electrochromatography (TLE).

The subject is enjoying something of a revival with significant advances having been made during the

1990s. If this trend continues, planar electrochromatography may take its place as a powerful tool in the modern analytical laboratory offering new modes of separation at speeds that are currently unavailable in conventional thin-layer chromatography (TLC).

Definitions

The term TLE will be used to refer to all techniques carried out within chromatographically active thin layers and with electric fields being employed to influence the separation. This will encompass a number of different modes of separation, including those that are dominated by electrophoretic analyte migration but which, due to the nature of the layer, include some element of chromatographic retention. Conventional gel-based electrophoresis is therefore distinguished from TLE by the electrophoresis in TLE being carried out in a chromatographically active layer of material such as silica, alumina or cellulose. TLE therefore excludes gel electrophoresis carried out in cast gel slabs of materials such as polyacrylamide that are commonly used for the separation of proteins and DNA. Paper electrophoresis is now largely obsolete and is not therefore covered.

Advantages of TLE

Thin-layer chromatography has proved to be one of the most successful chromatographic techniques ever devised. This success owes largely to its simplicity and versatility compared with column techniques. The main drawback of TLC is the low solvent velocity achieved through capillary action. This leads to long separation times and means that the optimum flow velocity (for chromatographic efficiency) is seldom reached. Electric fields can be used to cause the migration of the analyte, either by directly exerting a force on the molecules, or by causing the eluting solvent to flow, carrying with it the analyte. This can result in migration velocities of one to three orders of magnitude greater than those achieved through capillary action and can introduce an electrophoretic component to a separation, facilitating the separation of difficult mixtures.

Electrophoresis

This is the migration of ionized species under the influence of an electric field. Ions in an electric field experience a force proportional to their charge, causing them to accelerate in the direction of the field. Ions in solution, not interacting with a solid support, reach a terminal velocity dependent on the magnitude of the force and their interaction with the solvent. Therefore, different ions will migrate through the solvent at different rates. This can be used to separate different ions and is known as free solution electrophoresis.

If the solvent is within a bed of chromatographically active media such as silica, the migrating ions can interact with the solid support, adding a chromatographic component to the separation. At this point, it is simpler to refer to the separation as electrochromatography, which encompasses both electrophoretic and chromatographic effects.

The ionic mobility u can be expressed in terms of the ion's velocity v in a given electric field strength E by $u = v/\mathrm{E}$. This only applies to free solutions, since migration through the channels of a solid support follows a tortuous path that deviates from a straight line by a quantity dependent upon the properties of the solid support. The important point is that the migration velocity, both in free solution and through a bed of chromatographic media, is proportional to the potential applied. In order to achieve greater migration velocities, it is necessary to apply higher potentials.

Electroosmosis

Electroosmotic flow arises from the formation of an electrical double layer at a solid–liquid interface. This is due to the presence of charged species on the solid surface; either in the form of surface ionized groups (e.g. SiO^- in the case of silica) or because of the preferential adsorption of ions from the solution. In most cases it is a combination of both. The surface charges are counterbalanced by ions in solution, which form an immobile, strongly bound layer near the surface and a mobile, solvated layer extending into the liquid. Under the influence of an applied potential, the solvated layer of counterions moves, causing bulk solvent flow (**Figure 1**).

This means of inducing solvent flow has been successfully applied to capillary column chromatography, producing capillary electrochromatography (CEC). Using electroosmotic flow (EOF) to pump solvent through a column generates a 'plug' flow profile, which is distinct from the parabolic profile generated by hydraulic pumping. This results in reduced band broadening in CEC. It also allows the use of very fine chromatographic supports, which would be impossible to use in pressure-driven systems owing to back-pressure constraints. These factors combine to give high linear flow rates, of the order of $1\ \mathrm{mm\ s^{-1}}$, allowing very fast, efficient separations.

As with electrophoretic migration, the EOF velocity increases linearly with applied potential. This makes it generally desirable to apply higher potentials in order to achieve faster migration rates.

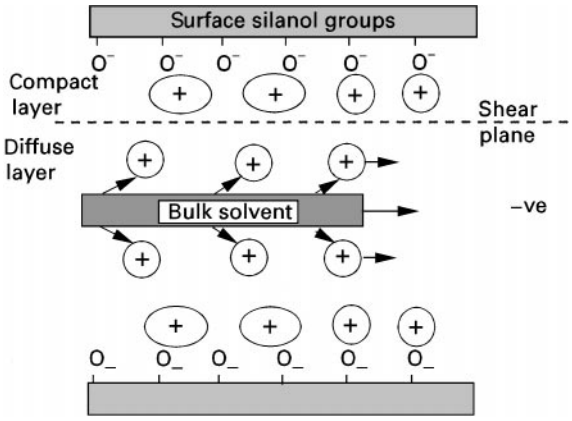

Figure 1 A schematic representation of double layer formation at a silica surface.

Modes of Migration

In standard TLC, the migration of a sample molecule is controlled by its interaction with the bed of chromatographic media and the partition of the solute into the eluting solvent. In electrically driven TLC, the solutes may be made to move in a number of different ways.

If the sample molecules are ionic in the solvent used, the application of an electric field will exert a force on them and they will migrate electrophoretically. As they migrate they are also subject to chromatographic partitioning between the solvent and the stationary phase. The individual components separate from each other by migrating at different velocities, each compound having a characteristic migration velocity that reflects the conflict between electrophoretic migration and chromatographic retention.

If, however, the sample molecules are uncharged in the solvent, they will only migrate if the solvent is made to flow. Whereas in standard TLC this is achieved by capillary action, in electrically driven TLC, with the right solvent and adsorbent, it can be achieved through EOF. The separation that results from an electroosmotically driven TLE experiment is, in the absence of electrophoretic effects, similar to that obtained by conventional TLC, but it is obtained much more rapidly.

There is a third mode by which solvent flow can be induced through a thin layer chromatographic media during an electrochromatography experiment. When a current flows through a wetted layer of chromatographic material the layer heats up. The power which has to be dissipated from the plate depends on the current flow and hence the resistance of the wetted plate. When the solvent is unevenly distributed through the plate this leads to an uneven evaporation of solvent from the plate causing capillary solvent migration to occur as a direct result of Joule heating. This effect is more extreme with vertically mounted plates, which under gravity drain solvent to the base of the plate. In some experiments, evaporative flow can be considerably larger than that which is generated by electroosmosis. This can be falsely identified as EOF and is particularly evident in experiments carried out with vertically mounted plates. In horizontally mounted plates, with a solvent reservoir at each end of the plate, solvent is replenished least quickly at the center of the plate. If the rate of evaporation is initially assumed to be uniform across the plate, then the middle of the plate will dry out more quickly. This leads to solvent flow from both ends of the plate towards the middle being superimposed upon any EOF.

The current flow through the solvent-wetted chromatographic layer is dependent on the overall electrical resistance of the plate, which is a function of the ionic density in the solvent. These ions may originate from soluble ionic species in the chromatographic material, dissolved ions in the solvent or dissociated solvent molecules. The smaller the ionic density, the higher the overall plate resistance and the smaller the current. Unless adequate cooling is provided, the input of power will cause a temperature rise in the thin layer. This will lead to evaporation of the solvent, the rate of which will depend upon the rate of power influx, the volatility of the solvent and degree of external cooling. This is the main limitation controlling the magnitude of the potential that can be employed in TLE to achieve faster migration rates.

Various methods of cooling the plates have been used in order to reduce solvent evaporation. Immersion of the plate in a solvent that is immiscible with the eluting solvent has been employed in various separations, with CCl_4 being the most popular coolant for aqueous eluent systems. The purpose of the solvent bath is to provide direct cooling to the plate surface. This approach was experimentally clumsy and limited the range of analytes, since analyte solubility in the 'coolant' must be considered. It was later dropped in favour of the use of cooling pads in contact with the TLE plate, achieving cooling rates in excess of 0.1 W cm^{-2}. With high-conductivity aqueous systems this arrangement allowed the applied potential to be raised to 160 V cm^{-1}, generating migration velocities of up to 0.1 mm s^{-1}.

Solvents with limited volatility, such as higher alcohols, propylene carbonate and formamides have been used to reduce evaporation. This approach did not however gain popularity owing to several experimental limitations, the most important of which is the difficulty in removing the eluting solvent from the

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chromatographic material following an experimental run. This is usually necessary in order to visualize the separated compounds.

Historical Development

The development of TLE occurred in tandem with that of paper electrophoresis (**Table 1**). This is not surprising, since both techniques require similar apparatus and reagents, and are generally used to achieve the same types of separation. TLE has always had an advantage over the paper technique in terms of chromatographic performance. The finer and more uniform surface structure achievable on thin layers results in considerably reduced band broadening when compared with fibrous media.

The earliest experiments were carried out in the 1940s using paper and layers of silica gel. A wide range of analytes was separated, largely employing aqueous systems. The high conductivity of the aqueous systems limited the applied potential to 10–50 $V\,cm^{-1}$ but the electrophoretic separations achieved at these potentials were still a considerable improvement on those obtained by the equivalent paper chromatography/TLC separations. The run times were typically of the order of 1–3 h, but some experiments, particularly protein separations, were run for as long as 24 h.

The development of gas chromatography (GC) and high-pressure liquid chromatography (HPLC) enabled high-efficiency separations to be achieved and diverted attention away from planar techniques. This led to thin-layer electrophoresis/electrochromatography being largely abandoned in the late 1960s in favour of the column techniques. Very few publications between 1970 and 1998 cite the use of TLE.

Following a period of active research into planar techniques between 1940 and 1960, interest in TLE has been sporadic at best. Long periods of inactivity have been punctuated by occasional reports of technical advances and/or applications of the technique. Possibly one of the most important of these is a paper by Pretorius *et al.*, which described very high-speed separations both on TLC plates and in columns utilizing EOF to mobilize solvent. This paper set a precedent, by using nonaqueous and low aqueous solvents, potential gradients of around 1000 $V\,cm^{-1}$ could be used – potentials at least five times greater than had been previously employed. The experiments carried out in columns were quickly followed up by several other research groups and are considered the direct predecessor of modern capillary electrophoresis (CE) and CEC.

Table 1 Developments in thin-layer electrochromatography

1937	Earliest recorded use of paper electrophoresis, separation of snake venom proteins followed by UV detection (Konig)
1946	Earliest recorded use of 'thin-layer' electrophoresis, in a slab of silica jelly. Method used for the separation of amino acids and peptides (Consden *et al.*)
1954	First use of electroosmotic flow as driving force to effect a separation. Polysaccharides separated on collodion membranes (Mould and Synge)
1961	Separation of amines and amino acids by thin-layer electrophoresis (Honnegar)
1963	Investigation of the characteristics of solution flow in thin-layer electrophoresis on a range of thin-layer chromatography (TLC) media. (Kowalczyk)
1974	High-speed separation of organic compounds on silica TLC plates and in columns in electric fields (Pretorius *et al.*)
1994	Planar electrochromatography on non-wetted thin layers (Pukl *et al.*)
1998	TLE separation of non-polar dyes on commercial reversed-phase TLC plates using electroosmotic flow (Nurok *et al.*)
1999	Quantification of electroosmotic and separation of basic pyrimidines by thin-layer electrochromatography (Howard and Shafik)

Experimental Techniques

Development Chambers

Historically, the majority of TLE experiments have been carried out in the horizontal mode, with solvent reservoirs at both ends of the plate. This set-up continues to be used today by several groups, and is shown in **Figure 2**. The plates are supported with the chromatographic surface either up or down.

Solvent is transported to and from the plates by wicks, which also serve as electrical contacts. A range of materials has been used as wicks, including filter paper, sintered glass and felt. The electrodes are usually submerged in the solvent and made of an inert conductive material, such as silver, stainless steel, carbon or platinum.

A less popular approach involves the use of vertical plates, with solvent at the base of the plate (**Figure 3**). Electrical contact is made via the solvent at the base

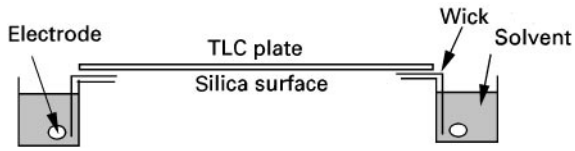

Figure 2 A horizontal tank design for thin-layer electrochromatography.

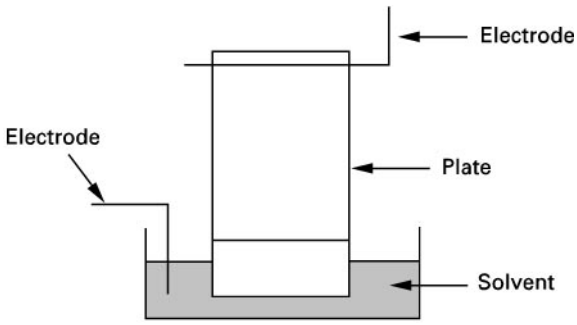

Figure 3 A vertical tank design for thin-layer electrochromatography with bottom solvent feed.

and an electrode fixed at the top of the plate. While in such systems the solvent is sometimes described as migrating up the plate because of electroosmosis, this may not always be strictly true. The main limitation of this arrangement stems from the lack of solvent reservoir at the top of the plate, and uneven solvent evaporation from the plate can impart a capillary-driven component to the solvent migration. This makes it difficult to differentiate between electroosmotic solvent flow and capillary solvent flow. Some workers have placed a second solvent reservoir at the top of the plate, and generated electroosmosis in a downward direction.

Chambers are usually sealed from the atmosphere in order to provide a solvent-saturated atmosphere, thus reducing evaporation. Some arrangements employ a cover plate, usually glass, in direct contact with the chromatographic surface in order to minimize evaporative effects.

Plates

A wide range of stationary phases, mobile phases and operating conditions have been employed in TLE. The layer is frequently 50–200 μm thick on a backing material of glass or organic polymer. Aluminium-backed plates are not suitable for use in TLE because of their electrical conductivity. TLE has been carried out on all thin layer adsorbants used for TLC, with silica, microcrystalline cellulose and alumina attracting the greatest interest.

Plates are generally 10–20 cm long and 5–20 cm wide. Longer plates tend to suffer from evaporative flow more than short ones and are generally avoided. The width of the plate is limited only by the current that the power supply is able to deliver at the required potential.

Solvent Systems

The majority of TLE separations have so far been carried out in aqueous buffer systems similar to those used in TLC. More recently, the use of nonaqueous systems has been shown to be useful in achieving faster and more efficient separations.

Potentials, Currents and Power Supplies

Potentials used in aqueous TLE are in the range of 10–100 V cm^{-1}, with currents of around 10–100 mA. This generates power levels of around 1–100 W. At the higher power levels, plate cooling is essential in order to prevent drying out. When nonaqueous systems are used, potentials between 200 and 2000 V cm^{-1} are used, with currents of 0.01–2 mA, generating between 0.02 and 20 W. Cooling of plates run at higher potential is seldom employed. Cooling is rarely necessary and very difficult to achieve due to the inherent incompatibility of high thermal conductivity and good electrical insulation characteristics in materials.

Starting Conditions

The layers are usually pre-wetted with the eluting solvent following sample application. This is achieved by spraying or dipping. Some experiments have been carried out using dry plates, but with limited success.

Sample Application and Visualization

The same methods of applying and viewing sample spots and bands used in TLC are employed in TLE.

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Applications

Thin layer electrochromatography can be divided into three main forms depending on the major factor governing the separation. While not mutually exclusive, since most separations include some element of the other modes, these broadly arise from electrophoretic solute migration, electroosmotic solvent flow and the natural spin-off from the heating effects arising from the applied potential, electrothermal elution.

The most commonly encountered examples of TLE are based around electrophoretic separations in aqueous solvent. Not surprisingly, given the historical success of paper electrophoresis, several workers have used thin layers of microcrystalline cellulose. In addition, cellulose acetate and silica have been used for the separation of proteins. Other applications have included the separation of starches, amino acids (and various derivatives) (**Figure 4**), organometallic compounds (**Figure 5**) and transition metal ions.

Electrophoretic separations are not limited to aqueous solvent systems and the higher resistance of nonaqueous solvents gives the advantage of lower currents and reduced heating effects. The separation

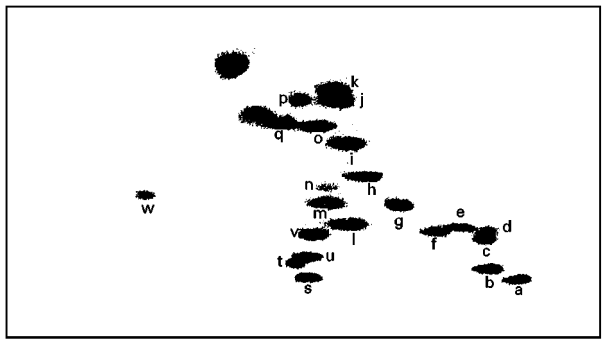

Figure 4 The separation of amino acids by two-dimensional thin layer electrophoresis–thin-layer chromatography with an aqueous electrolyte. The chromatographic media was plastic-backed cellulose layer. Electrophoresis in the first dimension using a 4%(v/v) aqueous formic acid electrolyte was followed by chromatographic elution in the second dimension with butanol-0.4%pyridineacetic acid (22 : 10 : 10, v/v/v). Adapted from E. McEvoy-Bowe (1985) *Journal of Chromatography* 347: 199–208, with permission.

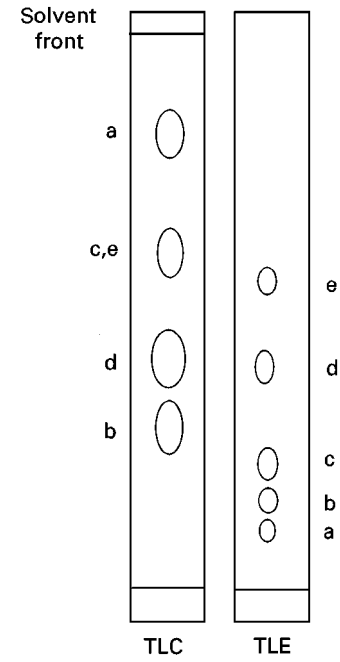

Figure 6 Nonaqueous thin-layer electrochromatography (TLE) and conventional thin-layer chromatography (TLC) of a dye mixture: (a) Oil Blue, (b) Rhodamine B, (c) Neutral Red, (d) Diazine Green, (e) Brilliant Green. The chromatography media was silica (electroosmotic flow suppressed) and the solvent was ethanol.

of a number of dyes using ethanol as the solvent is shown in **Figure 6**. In this separation electroosmotic flow effects were suppressed to reveal the electrophoretic migration of the charged dyes, resulting in completely different elution orders.

In TLE, solvent migration from EOF is easily confused with capillary-induced flow resulting from localized solvent evaporation. Broadly speaking, EOF is to be expected from wet polar solvents, protic solvents or from those that are capable of autoprotolysis.

With vertical tank systems, and particularly those employing nonpolar solvents, there must remain some uncertainty over whether thermal effects have been responsible for any solvent migration observed. This is the case in the pioneering planar systems studied by Pretorious (**Figure 7**), in which nonpolar solvents such as benzene were allegedly used. Our attempts to reproduce this work with a vertical tank system resulted in an electrically driven solvent

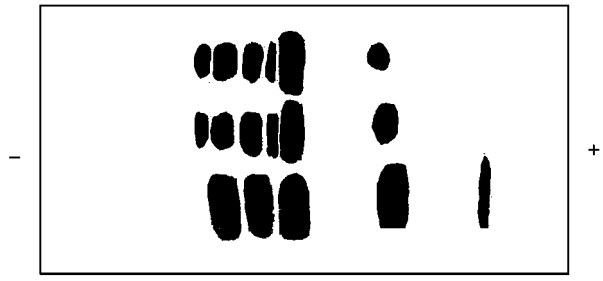

Figure 5 A thin-layer electropherogram of platinum chloroamine complexes. The chromatographic media was microcrystalline cellulose thin layers and electrolyte was 0.1 M $NaClO_4$, at 500 V for 5 min. Adapted from M Lederer and E Leipzig-Pagani (1998) *Analytica Chimica Acta* 358: 61–68, with permission.

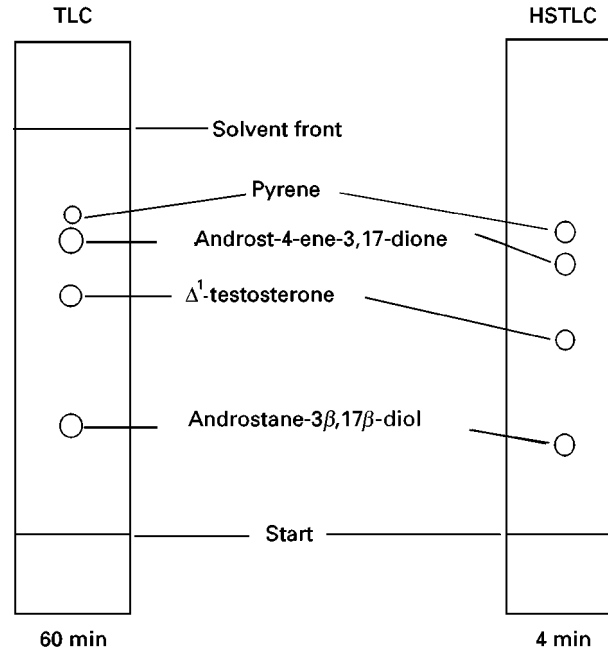

Figure 7 An early thin-layer electrochromatography (TLE) separation of nonionic compounds. The chromatography media was dichlorodimethylsilane-treated silica and the solvent was unspecified. Adapted from V. Pretorius *et al.* (1974) *Journal of Chromatography* 99: 23–30, with permission.

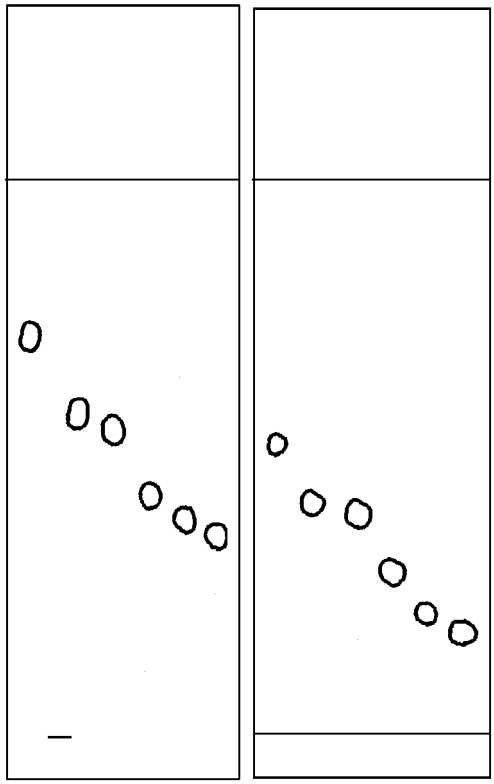

Figure 8 Conventional thin-layer (left) and electroosmotic (right) separation of pyrimidines employing identical silica layer chromatographic media and eluting solvent (ethanol) (TLC *ca.* 15 min; TLE 7 kV, 90 s). Adapted from AG Howard and T Shafik (1999) *Journal of Chromatography* 844A: 333–340, with permission.

migration and chromatographic separation resulting largely from thermal effects and not EOF. By changing to more polar solvents, in a horizontal tank, we have shown that true electroosmotic flow could be achieved. The separation of a number of pyrimidines on silica eluted with ethanol showed elution characteristics similar to those obtained by conventional TLC, but with higher separation efficiency and in one tenth of the time (**Figure 8**).

More recently, high-voltage nonaqueous TLE employing electroosmosis as the main driving force has been applied to the separation of a wide range of acidic, basic and neutral organic compounds, with considerable success.

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